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Moose Research Center Report

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**Research Performance Report
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This is a progress report on continuing research. Information may be refined at a later date.

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RESEARCH PROGRESS REPORT

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SUMMARY

We continued to collect baseline information on parameters of calving in nutritionally unrestricted caribou (*Rangifer tarandus*) for later comparison with nutritionally stressed animals. We also improved facilities and developed new methods for obtaining such information. Predictive equations of total body fat using ultrasonographic fat measurements were developed. A linear relationship exists between ingesta-free body fat and maximum rump fat thickness measured by ultrasonography. We also observed strong linear relationships between carcass and hide fat and ingesta-free body fat. By allowing a mature bull access to cows only during daylight hours of the rut, we were able to observe 6 planned breedings. Four calves (2 males, 2 females) were born and processed (weighed, measured, sexed, eartagged, and blood sampled) without loss. One old, but healthy, cow required human intervention to deliver a stillborn calf. A newly implemented annual vaccination schedule prevented further losses from *Chlostridium* spp. infections. Using a digital electronic scale, we weighed caribou intermittently, with minimal stress. Seven adult, 1 2-year-old, and 3 yearling females were immobilized during February–April 1998; Of these animals, 4 adults and 1 yearling tested were pregnant, based on serum assay. Mean ultrasonic rump fat thickness was 1.63 cm (\pm 0.23 SE) for pregnant caribou during late winter 1998. Mean gestation length was 228 (\pm 3.0 SE) days during 1997–1998. We continued monitoring 6 adult female caribou that used a restrictive feed gate system. The Moose Research Center (MRC) caribou herd comprises 15 animals.

Key words: .Body condition, caribou, gestation, nutrition, *Rangifer tarandus*, reproduction.

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Inside front and back covers: Overview of the Moose Research Center

BACKGROUND

Data from the Southern Alaska Peninsula caribou herd (SAP) indicate a declining population, small adult body size, low birth weights, late calving dates, and low calf survival. Undernutrition is the suspected agent affecting the population dynamics of that herd (Pitcher et al. 1991). Post (1995) recently confirmed food limitation (i.e., lichen availability) as a factor in the decline of the SAP herd. In addition, differences in spring plant phenology were also important in determining calf productivity. Adams (1996) documented an interaction among forage resources, climatic conditions, and previous reproductive success in determining the productivity of female caribou in Denali National Park.

Body condition of adult female caribou and reindeer affects reproductive performance and calf survival. Lenvik (1988) found that conception date in reindeer was related to weight (and possibly energy reserves) of females during the breeding season. Pregnancy rate was closely associated with fat reserves and body weights of Peary caribou in Arctic Canada (Thomas 1982). Calves of undernourished female reindeer had reduced birth weights and reduced survival (Espmark 1980, Skogland 1984). Gerhart (1995) determined that body fat content was the body condition parameter that best explained differences in pregnancy rate in Porcupine Herd caribou.

Experiments on winter protein metabolism in caribou and reindeer have involved primarily nonreproductive animals. Allys-Chan (1991) examined the effects of summer dietary protein on body reserves and milk production in caribou. Case (1996) reviewed the extreme ability of caribou to recycle nitrogen. Although caribou possess the ability to conserve nitrogen in the context of survival, less is known about requirements for reproduction. Although common winter caribou feeds such as lichen are generally adequate in providing energy, they often contain minimal protein. Excessive protein in the diets of ruminants has resulted in decreased reproductive efficiency (Ferguson and Chalupa 1989) and has been negatively correlated with fat deposition (Holter and Hayes 1977). Allys-Chan (1991) failed to observe an effect of differing protein levels (10% and 20%) on fat or protein deposition in adult female caribou during summer. However, both of these levels may have exceeded requirements for protein synthesis in caribou. She did observe differences in the ability of lactating and nonlactating females to deposit fat reserves during summer.

Undernutrition of *Rangifer* females during gestation and possibly before breeding resulted in late calving (Espmark 1980, Reimers et al. 1983, Skogland 1984). Late calving reduces the summer growth season during the first year (Klein et al. 1987) and probably reduces survival of calves into the following winter (Haukioja and Salovaara 1978). For caribou there are strong indications that nutrition, growth, condition, productivity, and survival are linked; however, our knowledge of these relationships is incomplete and additional information is needed to guide management.

OBJECTIVE

Determine the effects of nutrition on breeding chronology, calving chronology, gestation length, birth size, neonatal survival, and maternal body condition. We wish to refine the relationship between nutrition and previous reproductive success in determining future reproductive success. Because of the potential for protein, as well as energy deficiencies in wild caribou, the role of both nutrients will be further explored, particularly with regard to the relative importance of seasonal ranges.

STUDY AREA

Research was conducted at the Kenai Moose Research Center, Soldotna, Alaska.

METHODS

During July 1997–June 1998, we fed caribou an ad libitum textured reindeer ration (16% crude protein, 5.5% crude fiber) during all months. During July 1996–November 1996, captive caribou at the MRC were switched to the ad libitum textured reindeer ration. During November 1996–January 1997, we fed caribou a mixture of the 16% textured ration and the 13% pelleted ration. During January–April 1997, caribou were fed the 13 % pelleted ration. Animals were returned to the 16% ration in May 1997 to simulate a higher quality natural summer diet.

Previously, during May and June 1995–July 1996, we provided captive caribou at the MRC an ad libitum pelleted reindeer ration (13% crude protein, 15% crude fiber). Prior to this, animals were fed a 1:1 ratio of pelleted moose ration (10% crude protein, 5% crude fiber; Schwartz et al. 1985)

and a different pelleted reindeer ration (16% crude protein), periodically supplemented with alfalfa hay. Caribou were confined to a 4-ha enclosure with access to additional grasses (*Calamagrostis* spp.) and forbs.

Weights of caribou older than neonates were obtained intermittently (because of facility remodeling) using a 12-volt electronic platform scale (Tru-Test Limited Model 700, Auckland, New Zealand). During 25 February–22 April 1998, 3 adult, 2 2-year-old, and 1 yearling female were immobilized with a carfentanil citrate/ xylazine hydrochloride mixture and reversed with Yohimbine and Naltrexone. We attached individual-specific “keys” for the self-feeding gates, using standard domestic dog collars. Serum was collected by jugular venipuncture and frozen (-20 C) for eventual pregnancy-specific protein B assay (Stephenson et al. 1995) to diagnose pregnancy. Portable real-time ultrasound was evaluated for measuring lipid reserves (Stephenson 1995). The rump region was scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Conn., USA) with a 5-Mhz, 8-cm linear-array transducer. Subcutaneous fat thickness was measured along a line between the spine, at its closest point to the tuber coxae (hip bone) and the tuber ischii (pin bone). Fat thickness was measured with electronic calipers to the nearest 0.1 cm at the midpoint and point of maximum thickness (immediately adjacent to the cranial process of the ischial tuber) along the line. We measured thickness of the Longissimus dorsi, caudal to the last rib, and the Biceps femoris, beneath the midpoint fat thickness, using ultrasound as potential indicators of protein reserves.

When possible, body composition determination was conducted for fresh mortalities as follows. We determined whole body mass and then each animal was eviscerated and skinned (subcutaneous fat remained on the carcass). The carcass was bisected longitudinally along the vertebral column, with one half frozen for chemical analysis. The gastrointestinal tract was emptied of ingesta. The concepta and amniotic fluid of pregnant females were removed and their mass determined to permit calculations less the concepta. Kidney fat mass was recorded as the mass, to the nearest 1-g of trimmed fat attached to the kidney (Riney 1955). The entire viscera and samples of shaved hide were frozen for analysis. The frozen carcass half and visceral mass were sliced at 51- and 25-mm intervals, respectively, on a commercial band saw (Huot and Picard 1988). The homogenate at the base of the blade was collected for each component, mixed, and refrozen. Hide samples were freeze-dried and ground in a Wiley mill to create a homogenate. Chemical analysis of frozen samples was conducted at Washington State University's Wildlife Habitat Laboratory. Crude fat was determined by ether extraction (AOAC 1975). Samples were analyzed in duplicate.

Percent fat of a body component (e.g., carcass, viscera, hide) refers to its chemical determination. Values for viscera exclude ingesta and uterine contents. Percent ingesta-free body fat (IFBFAT) was calculated by summing the products of each component's percent fat and its respective mass, dividing by ingesta-free body mass, and multiplying by 100.

We weighed newborn calves, using a spring scale (Salter No.235, London, England), eartagged, sexed, and measured for total length, jaw length, hind foot length 1 (metatarsus), and hind foot length 2 (heel to toe). In addition, blood (5 cc) was collected by cephalic venipuncture using a syringe. We processed calves within 12–24 hours after birth. Serum samples were analyzed at Phoenix Central Laboratories, Seattle, Washington, USA.

During the 1997 breeding season, 7 females were confined in a 50 x 50-m enclosure. To ensure that breeding dates were known, a 4-year-old male was allowed access to the females during a 1-hour period in the morning and evening and observed.

We tested a controlled-access feeding system (American Calan, Inc., Northwood, NH, USA) for suitability with caribou. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing "key" collar worn by the animal (Mazaika et al. 1988). Animals were trained to use the gate feeders in pairs by initially unlocking the 2 gates specific to their keys and propping the gates open for 1 day. The following day gates were closed but remained unlocked. During subsequent days, gates were locked for both individuals, and animals were required to use their "keys" to access feed.

In preparation for feeding trials to be conducted this winter, we are remodeling our handling facility to better accommodate caribou. In particular, we are constructing a more efficient weighing and immobilization facility. In conjunction with Calan feed gates, we built an additional enclosure for confinement of animals in feeding trials.

RESULTS AND DISCUSSION

We observed copulations for 5 confined adult females and 1 yearling during the presumptive first estrus. We determined the 1 yearling and 5 adult cows to be pregnant by pregnancy-specific protein B assay during February–April. The entire herd was vaccinated against *Chlostridium* spp. (*C. perfringens* and *C. sordellii* were cultured from tissue collected from MRC caribou) and wormed with Ivermectin® during winter 1997–1998. Four females determined to be pregnant calved successfully in May. Mean ultrasonic rump fat thickness was 1.63 cm (\pm 0.23 SE) for pregnant caribou during 1998 (Table 1). In contrast, during 1996 and 1997 rump fat thickness averaged 1.08 cm and 2.81 cm, respectively, for pregnant cows. No adult animals exhibited reproductive pauses. Gerhart (1995) suggested that lactational infertility can occur regardless of maternal fat reserves, particularly for cows that lactate well into autumn. Cameron (1994) suggested that periodic reproductive pauses were a response by caribou to nutritional stress. The improved nutritional quality of diets provided by feeding the textured ration during the last 2 years seems to have increased fat deposition and possibly reduced reproductive failure at the MRC.

Upon initial arrival of caribou at the MRC, we began feeding an experimental pelleted 16% crude protein reindeer ration developed by the University of Alaska, Fairbanks. However, because our animals developed scours, we blended this ration 1:1 with a 10% crude protein pelleted moose ration. We fed this diet during 1992–1995. During May–June 1995, we began using a 13% crude protein pelleted reindeer ration. However, although MRC captive caribou were intended to be on a high nutritional plane, the poor reproductive performance observed during 1995–1996 indicates that our caribou were on a less-than-optimal nutritional plane. Diet analysis of free-ranging caribou in Interior Alaska indicates a summer diet high in crude protein and digestible energy (Boertje 1985, 1990). Currently, animals are fed a 16% textured ration throughout the year.

One female and 3 males were born during 1998 (Table 2). Mean gestation length in 1996–1997 was 228 days (range 222–235). Mean gestation length for all previous years ($n = 18$) was $(223 \pm 3.7 \text{ SD})$ days (range 215–231); the median and mode were 224 days. Mean calf mass at birth was 9.7 kg (range 7.3–11.4) which was higher than that observed in any Alaskan free-ranging herds (Valkenburg 1997).

Cementum tooth analysis provided accurate age estimates for 4 of the original caribou that were introduced to the MRC. BY and BR were both 18 years at death; White and Orange were 9 and 8 years, respectively. Age may well explain the reproductive failure observed in BY during the last 2 years; however, BR never ceased reproducing.

There were strong linear relationships between IFBFAT and both carcass ($r^2 = 0.98$) hide ($r^2 = 0.97$) fat (Fig. 1 and 2). Surprisingly, visceral fat exhibited a weaker relationship than expected with IFBFAT ($r^2 = 0.30$; Fig. 3). Maximum rump fat thickness (MAXFAT) and SUMFAT (sum of the midpoint and maximum thickness) both were linearly related ($r^2 = 0.55$ and 0.57) to IFBFAT (Fig. 4 and 5). However, there was a much stronger relationship ($r^2 = 0.98$) between MAXFAT and IFBFAT upon exclusion of 1 outlying animal (Fig. 6). Because of this small initial sample size, further samples will be needed to further validate the predictive equations. Using data from captive MRC caribou handled during 1996–1998, we observed a weak relationship ($r^2 = 0.34$) between body mass and ultrasonic rump fat.

Although Allye-Chan (1991) did not calculate slopes, she presented plotted data on back fat and total body fat that appeared similar to our limited sample. Although the general slopes were similar, she observed low levels of back fat using dissection techniques at low total body fat. She suggested lipolytic animals in late spring possessed little to no subcutaneous fat regardless of total body fat. Our data do not support such a conclusion based on photoperiod.

RECOMMENDATIONS

In feeding trials this winter, we will focus on refining the relative roles of protein and energy in body reserve deposition and on the prediction of these reserves. Diets will be manipulated to simulate varying season lengths and diet quality.

ACKNOWLEDGMENTS

We thank S. Rickabaugh, S. Johns, and O. Ormseth for assistance with animal handling and facility remodeling.

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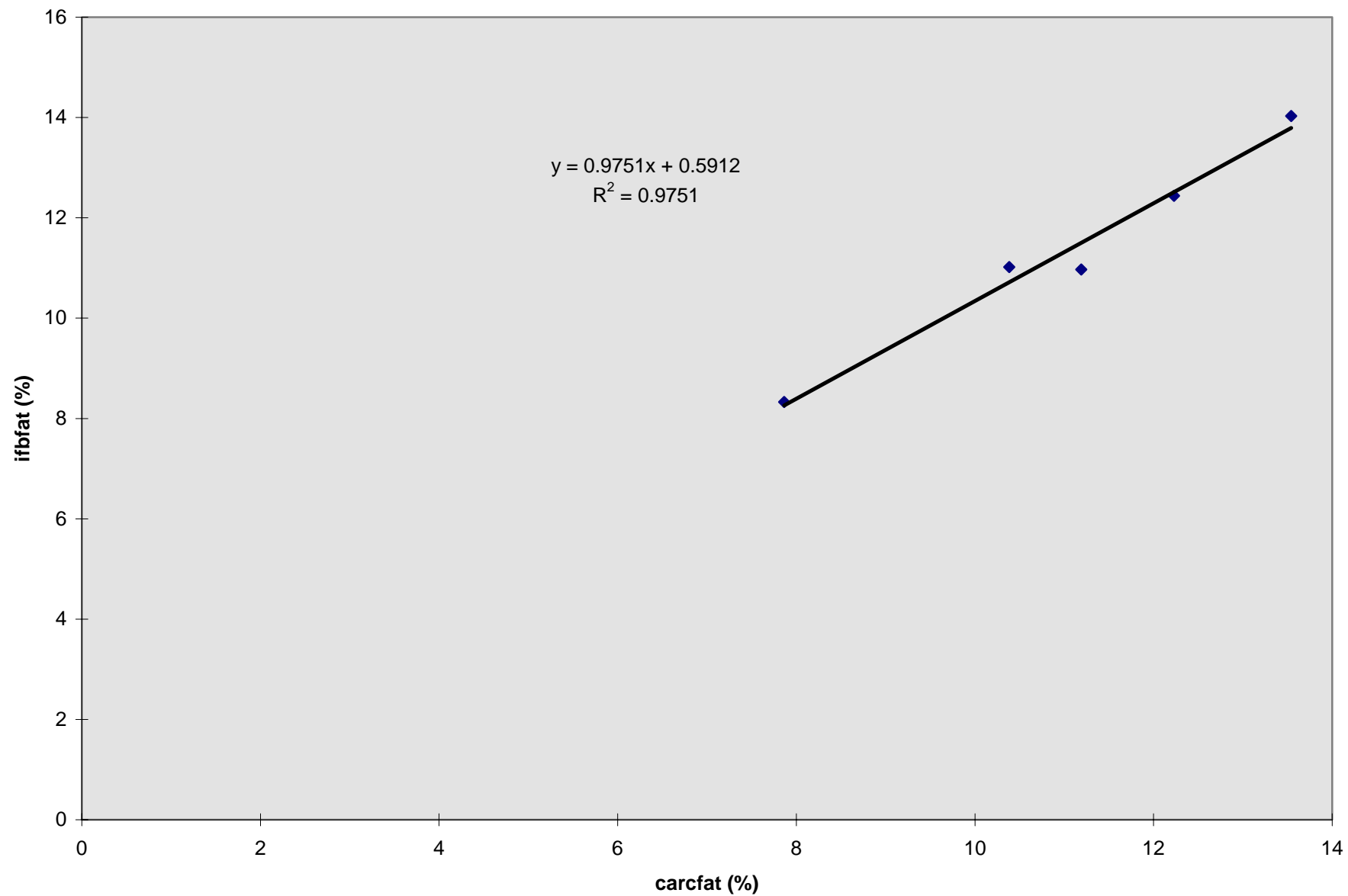
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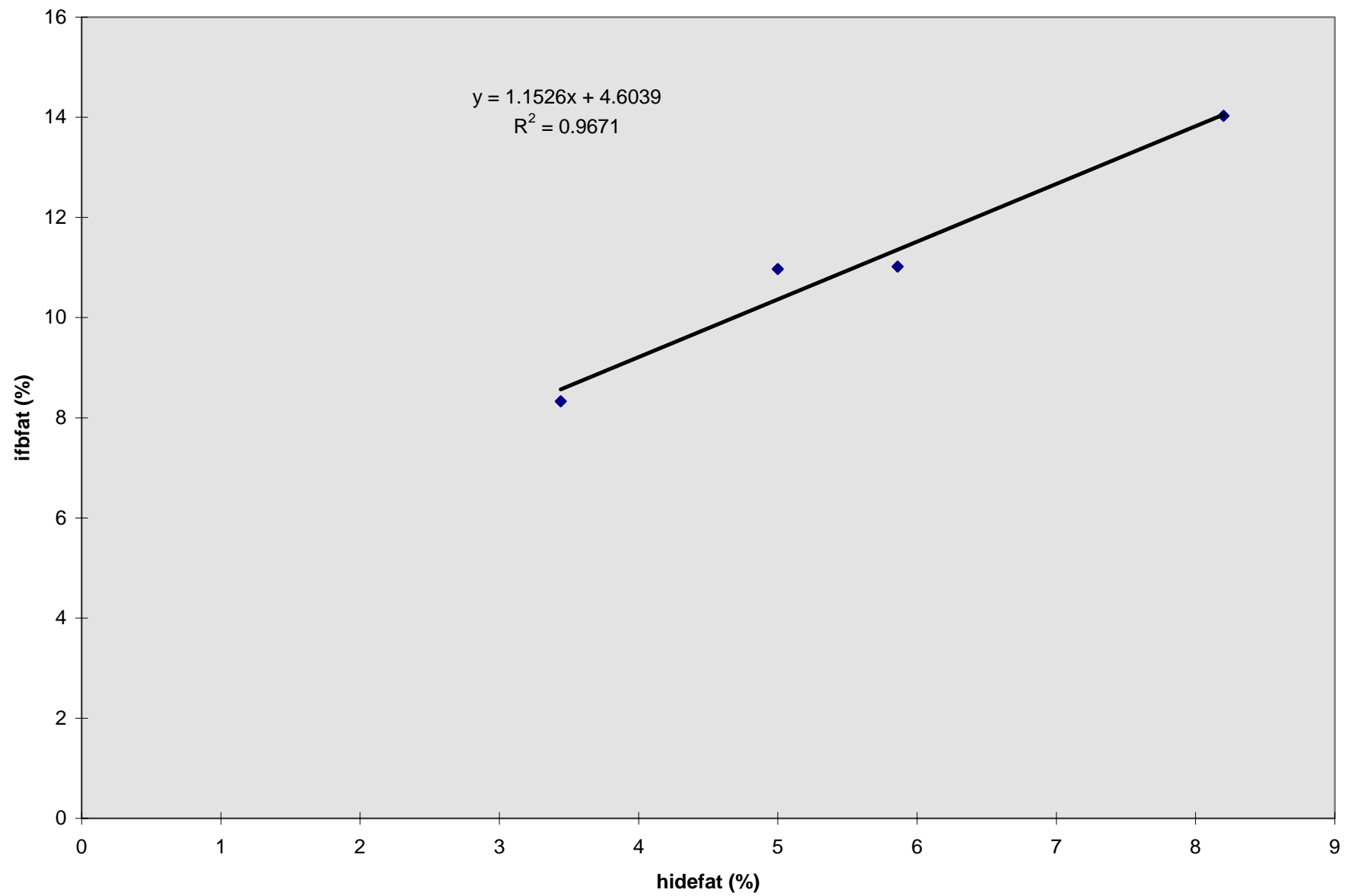
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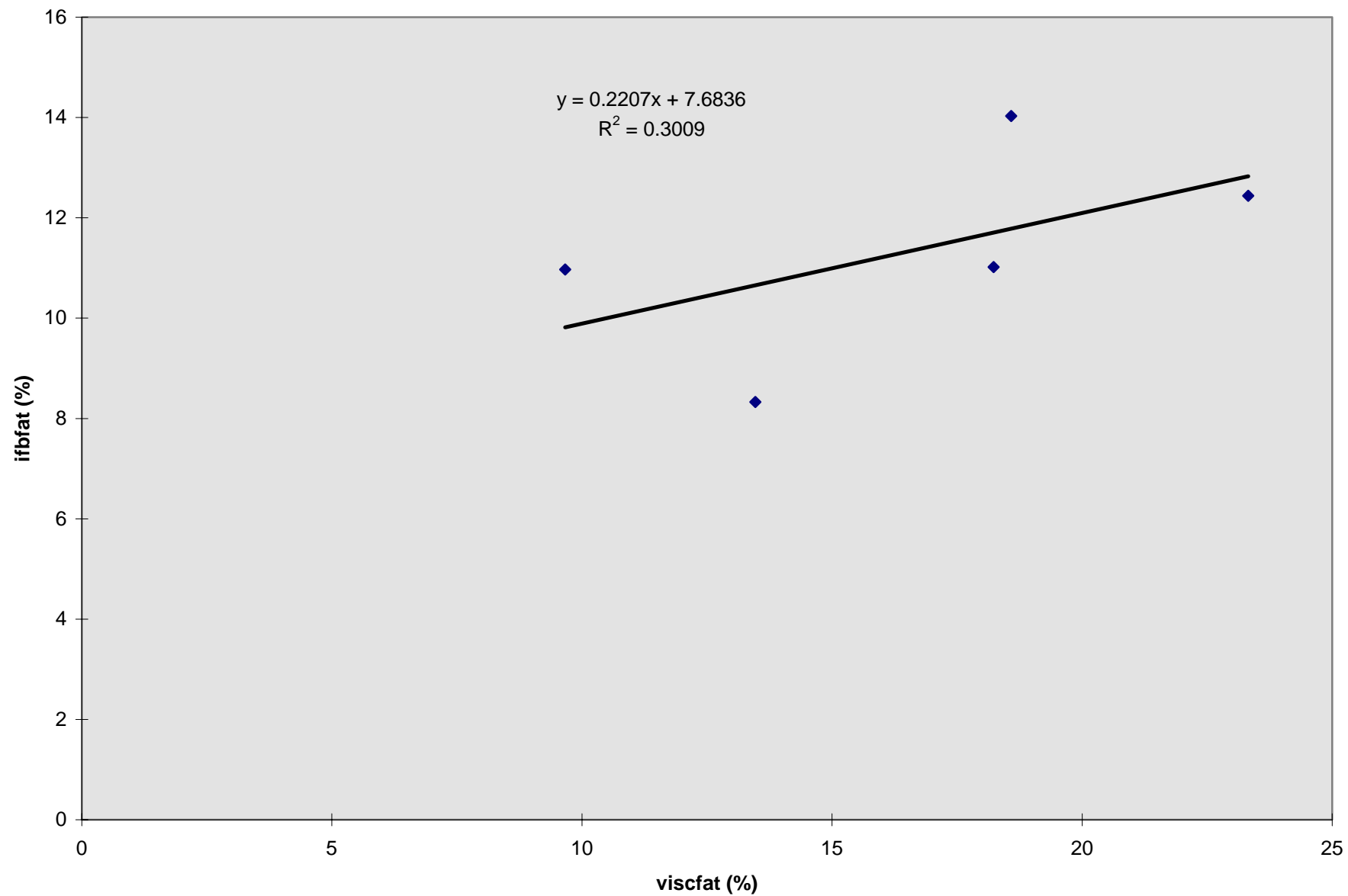
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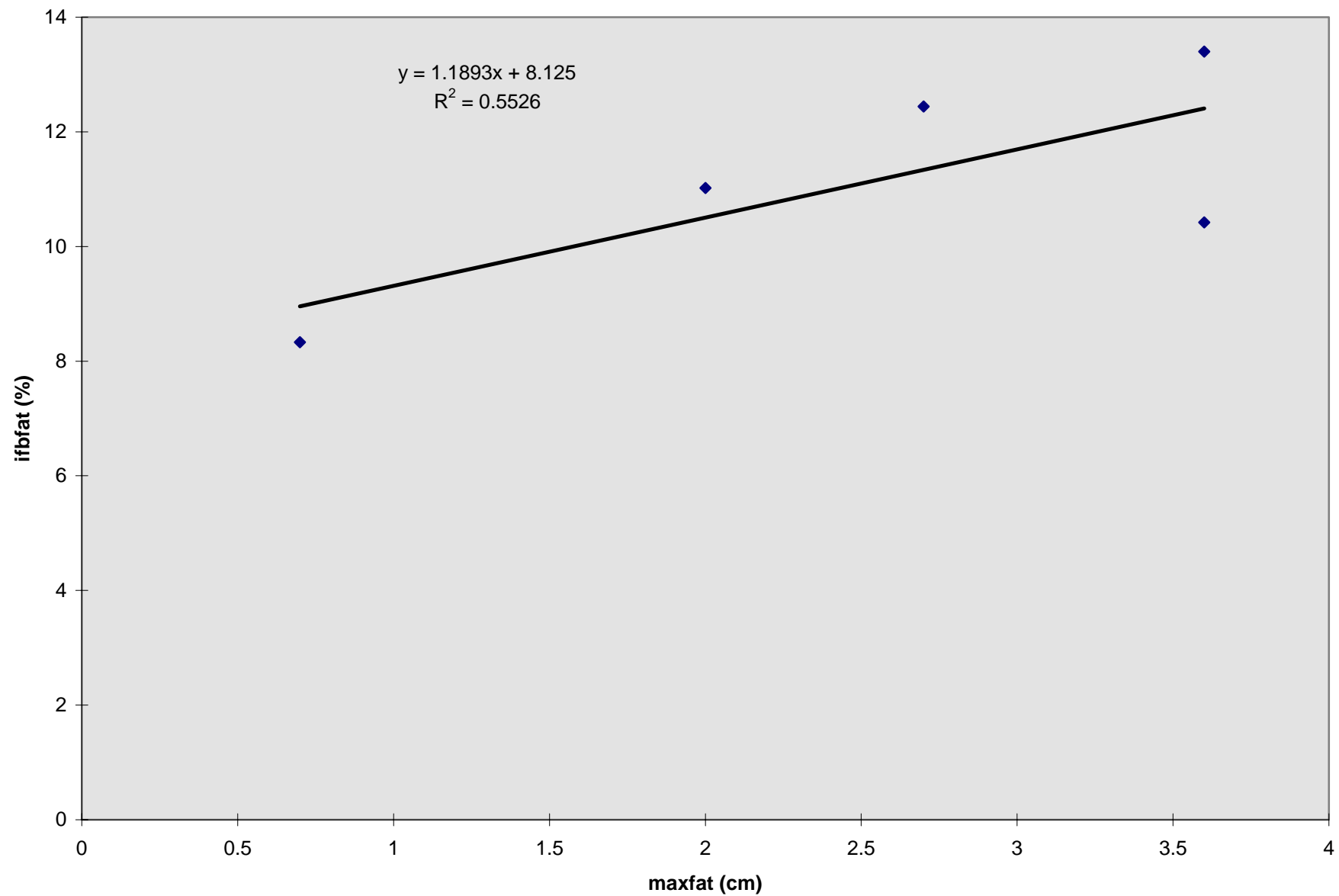
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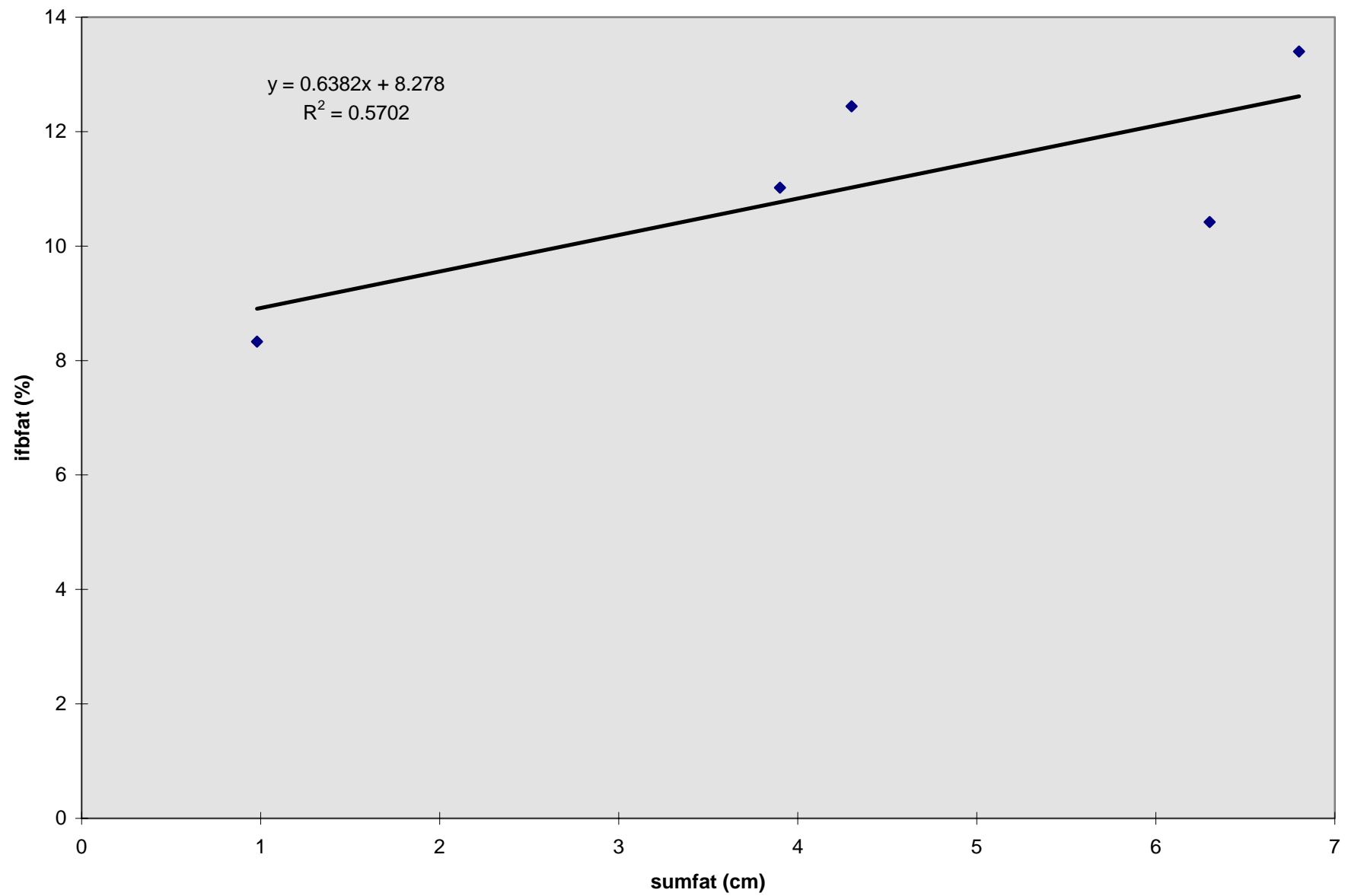
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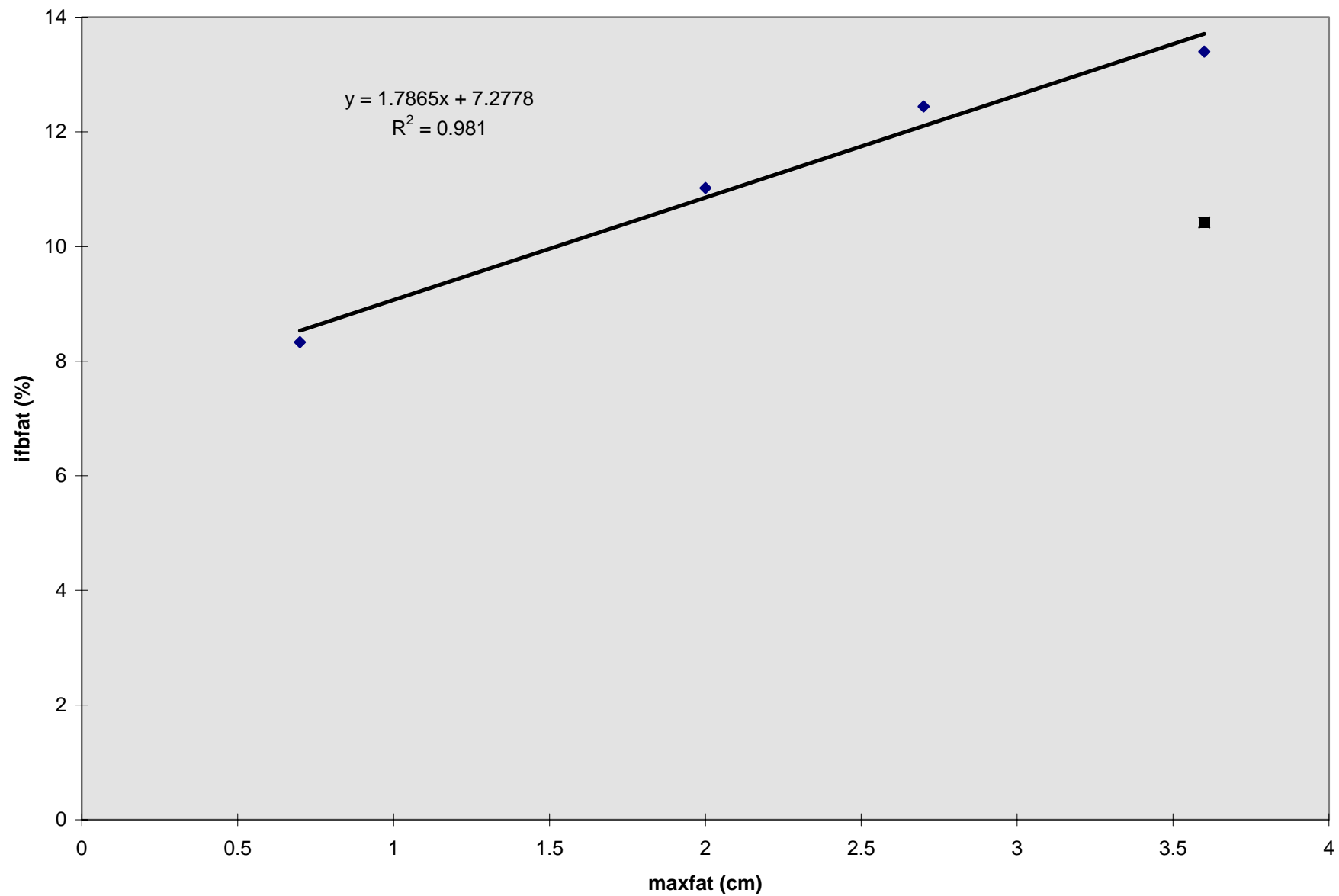












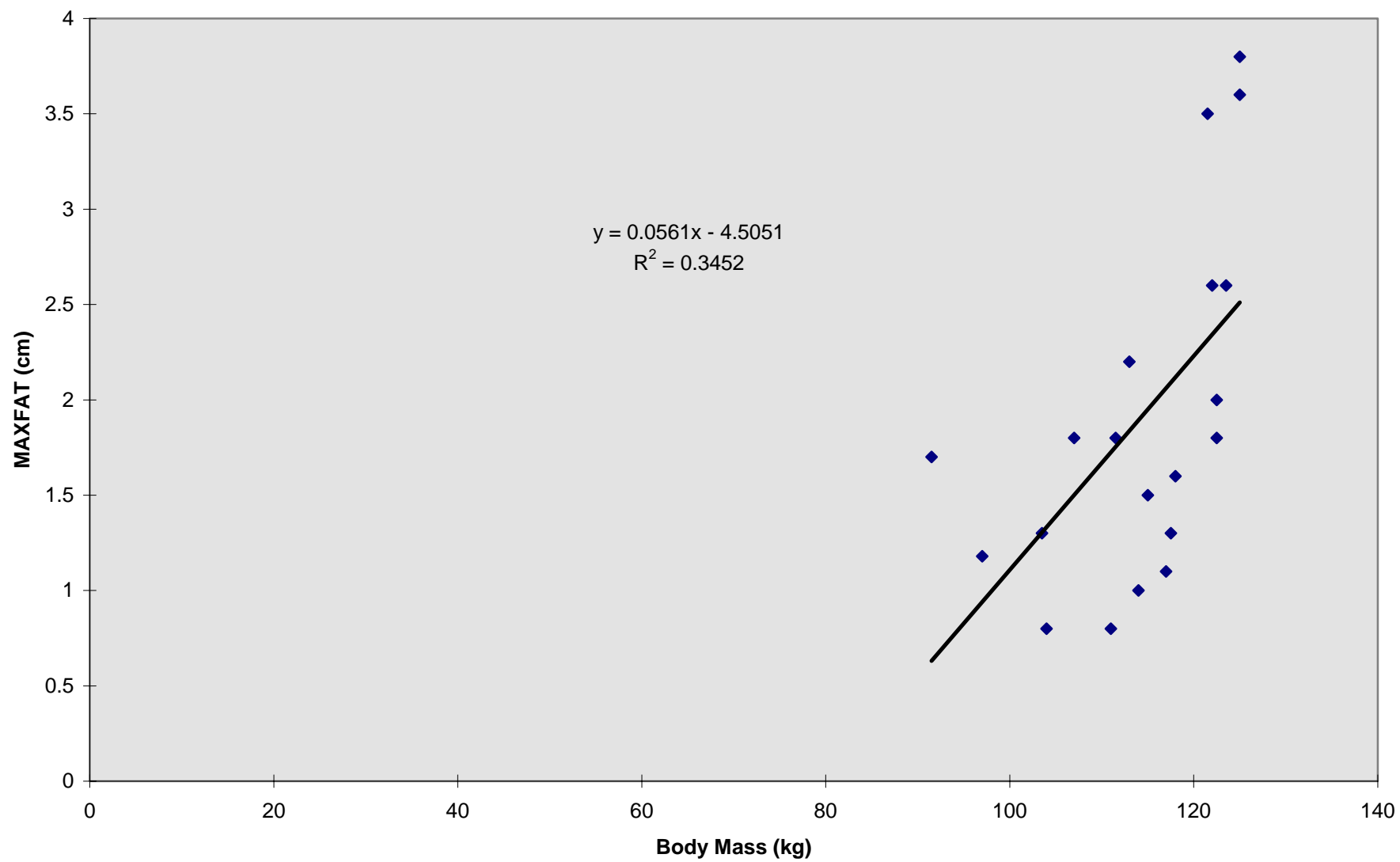


Table 1 Physical and physiological characteristics of caribou immobilized at the Moose Research Center, 25 February 1997–22 April 1998

Animal	Age (years)	Sex	Weight (kg)	Capture Date	Rump Fat thickness (cm) ¹	Biceps femoris thickness (cm)	L. dorsi thickness	Pregnant ²
Blue	≥12	F	122.5	4/22/98	1.8			Yes
Crystal	2	F	117.5	2/28/98	1.3	3.22	4.60	Yes
Jade	2	F	111.5	2/25/98	1.8	1.63	4.08	Yes
Orange	8	F	117.0	3/8/98	1.1	1.7	3.7	Yes
Violet	5	F	122.0	2/28/98	2.6	2.1	4.48	Yes
Mindy	1	F	97	2/25/98	1.18	1.88	5.21	Yes

¹Maximum rump fat thickness measured by ultrasonography.

²Pregnancy status determined by radio-immuno assay for Pregnancy-specific Protein B and confirmed by parturition or necropsy.

Table 2 Descriptive data for caribou calves captured within 24 hours of birth at the Moose Research Center, 25 May–11 June 1998

Animal	Sex	Date		Gestation length (days)	Birth weight (kg)	Total length (cm)	Mandible length (cm)	Metatarsus length ¹ (cm)	Hind length ² (cm)	foot Dam	Sire
		Conception	Birth								
9801R	F	12 Oct 97	May 98		11.4					Blue	Hebou
9802R	M	14 Oct 97	25 May 98	224	10.9	91		30	38	Violet	Hebou
9803R	F	21 Oct 97	30 May 98	222	7.3	86		25	33.5	Mindy	Hebou
9804R	M	20 Oct 97	6 June 98	230	10.7	89		28	36	Jade	Hebou
9805	M	20 Oct 97	11 June 98	235	8.2	80	14.5	25.5	31.5	Crystal	Hebou

¹Length of the hind foot minus the hoof.

²Length of the hind foot to tip of hoof.

RESEARCH PROGRESS REPORT

STATE: Alaska

STUDY: 1.52

COOPERATORS: Kenai National Wildlife Refuge, Soldotna, Alaska

GRANT: W-27-1

TITLE: Physiological Ecology of Moose: Nutritional Requirements for Reproduction with Respect to Body Condition Thresholds

AUTHORS: Thomas R. Stephenson, Kris J. Hundertmark, and John A. Crouse

PERIOD: 1 July 1997–30 June 1998

SUMMARY

We conducted feeding trials with adult female moose (*Alces alces*) on high and low quality diets to assess the influence of nutrition and reproductive success on body condition and future reproduction. Although dry matter intake rates did not differ between animals on the 2 treatments, metabolizable energy intake was reduced for the low quality diet and was reflected in greater loss of body fat and body mass. We measured daily mass gain in a sample of calves during the first 7–14 days postpartum. An unanticipated vitamin E deficiency resulted in high rates of abortion, stillbirths, and white muscle disease in neonates. We participated in development of a quantitative PSPB test, using blood serum that predicts twinning in utero with >90% accuracy. We compared body condition and in utero litter sizes among free-ranging populations within the state of Alaska in an effort to compare habitat quality and density effects among statewide moose populations.

Key words: *Alces alces*, energy intake, body condition, fat reserves, reproduction, ultrasound, PSPB, selenium, vitamin E.

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BACKGROUND

To facilitate intensive management of moose populations, we must be able to predict survival and reproductive success of individuals within these populations. Although population size is dictated by numerous factors such as weather and predation, ultimately habitat quality defined by the nutritional quality of diets will determine the maximum number of moose that an area can support. Reproductive performance of cow moose is related to their body condition. We intend to

refine the use of an individual animal's condition as an indicator of the nutritional quality of its habitat and as a predictor of its potential for reproduction and survival.

Recently, methodology for applying the "animal indicator concept" (Franzmann 1985) was validated. Stephenson et al. (1998) developed equations to predict total body fat in moose from ultrasonographic fat measurements. Hundertmark et al. (1994) also developed equations to predict body composition using bioelectrical impedance analysis. The animal indicator approach assumes that because the animal is a product of its environment, it represents the quality of its environment. Thus, rather than define carrying capacity in numbers of animals, this approach provides a relative indication of the proximity of the population to K. Recently, Grubb (1995) defined nutritional condition as "the state of body components controlled by nutrition and which in turn influence an animal's fitness." Saltz et al. (1995) noted that Grubb's definition clearly identifies the role of nutrition in determining an animal's condition and ultimately its reproductive success.

Because body fat is the primary energy store of the body (Price and White 1985), measurement of lipid reserves has been the focus of much research aimed at estimating nutritional condition (Stephenson et al. 1998, Chan-McLeod et al. 1995, Franzmann and Ballard 1993, Harder and Kirkpatrick 1994, Gerhart 1995). Assessment of body condition provides insight into the ability of individuals in a population to survive and reproduce. However, in order to evaluate the role of body condition in determining an animal's reproductive fitness, we also must be able to assess reproductive performance including ovulation, conception, fetal numbers and survival, and natal survival.

Although summer twinning rates have been used to indicate the quality of moose habitats (Franzmann and Schwartz 1985), undetected predation may lead to biased postpartum estimates (Stephenson et al. 1995). Knowledge of reproductive status is critical to understanding both reproductive performance and the costs of reproduction. Ultrasonography has been used to successfully determine in utero pregnancy and twinning in moose during both early (Stephenson et al. 1995) and late gestation (Testa and Adams, in press). Because ultrasonography requires specialized equipment and expertise, a serum assay that diagnoses twinning is of interest. Willard et al. (1995) recently developed a quantitative pregnancy-specific protein B assay for domestic sheep that permitted detection of fetal twins with up to 82% accuracy.

Although the existence of threshold "set points" of body condition have been hypothesized for ungulates (Schwartz et al. 1988; Renecker and Samuel 1991; Gerhart 1995), their existence relative to reproduction in moose has only recently begun to be quantified (Sand 1998, Testa and Adams, in press, M. Keech, unpubl. data). An understanding of thresholds required for ovulation, gestation, neonatal calf survival and identifying the mechanisms of reproductive failure will enhance our insight into the importance of different seasonal habitats and the management of these habitats.

Poor maternal nutrition may lead to failure in the passive immunity process between mother and offspring and increase susceptibility to diarrhea, septicemia, and other diseases in neonates. Sams et al. (1996) identified a relationship between serum immune parameters and neonatal mortality of white-tailed deer fawns. Low neonate serum levels of colostral antibodies may occur from

inability to efficiently nurse, poor colostral absorption, or depressed colostrum production (Sams et al. 1996). Indices of fawn viability such as immunocompetency or maternal condition may provide insight relative to the additive or compensatory nature of predation.

To validate the animal condition approach and define density effects on body condition and reproduction, we will conduct experiments with animals foraging on natural browse in addition to animals on trials using pelleted rations. As a population approaches carrying capacity, increased competition for forage resources should reduce average body condition. Hobbs and Swift (1985) hypothesized that as population density increases, the upper limit on nutritional quality of diets obtainable will progressively decline. Deterioration in the nutritional status of individuals would be expected as population density increases. The condition of individuals could be monitored to assess diet quality. However, ruminants may be able to increase intake rate in response to declining forage quality. Determining the ability of moose to compensate as density changes will enable us to understand the limitations of using the animal condition approach to assess habitat quality and the mechanisms of density dependence.

OBJECTIVES

- 1 Determine overwinter nutritional requirements for reproductive success in female moose.
- 2 Determine thresholds in body condition at which reproductive performance declines.
- 3 Evaluate the existence of cumulative effects in female moose relative to body condition, reproductive performance, and nutrition.
- 4 Refine estimation of moose body composition.
- 5 Using ultrasonography and a quantitative serum assay, develop and refine methodology for diagnosing twinning in moose.
- 6 Evaluate effects of density dependence on body condition, reproductive performance, and diet quality of moose on natural browse.

STUDY AREA

This research was conducted at the Moose Research Center located on the Kenai Peninsula, Alaska (60°N, 150°W).

METHODS

JOB 1 CONDUCT FEEDING TRIALS TO EVALUATE THE RELATIONSHIP BETWEEN MOOSE NUTRITION, BODY CONDITION, AND REPRODUCTIVE PERFORMANCE

Ten adult female moose were randomly assigned to 1 of 2 treatment groups (5 per group). Treatment groups consist of a high quality pelleted moose feed (Schwartz et al. 1985) and a poorer quality submaintenance ration developed during this study (Tables 1, 2). During January–April 1998, rations were offered ad libitum. Animals, confined together in a 4-ha fenced

enclosure, accessed feed, using individual-specific feed gates (American Calan, Inc., Northwood, New Hampshire USA) developed for controlled-access feeding trials. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing “key” collar worn by the animal (Mazaika et al. 1988). Known amounts of feed were offered and orts were collected daily to permit calculation of daily energy and protein intake for each animal. Subsamples of feed offered and orts were collected daily and frozen for subsequent dry matter determination. Trials were conducted during 1 January–30 April 1998. During October–December 1997 and May 1998, animals were maintained on the high quality pelleted ration ad libitum. During the remainder of the year (June–September), animals were maintained on natural browse.

We immobilized moose during September, November, January, March, and April, using carfentanil hydrochloride/xylazine hydrochloride, reversed with naltrexone/tolazoline. Portable, real-time ultrasound was used to measure fat reserves of adult females. The rump region was scanned using an Aloka model 500 ultrasound device (Aloka, Inc., Wallingford, Connecticut USA) with a 5-MHz 8-cm linear-array transducer. Ultrasonic fat thickness was measured at 2 sites along a line between the spine, at its closest point to the coxal tuber (hip bone), and the ischial tuber (pin bone). Subcutaneous fat thickness was measured with electronic calipers to the nearest 0.1 cm at the midpoint and point of maximum thickness (immediately adjacent to the cranial process of the ischial tuber) along the line. Two fat thickness indices were determined: 1) the maximum fat thickness detected along the line (MAXFAT), and 2) the sum of the maximum thickness and the thickness at the midpoint (SUMFAT). Stephenson et al. (in press) developed an equation to predict percent ingesta-free body fat from rump fat thickness ($R^2 = 0.96$, $SEE = 1.09$). Ingesta-free body fat was calculated using the equation: ingesta-free body fat (%) = $5.61 + 2.05$ (maximum fat thickness).

In addition, moose were weighed in September and weekly during feeding trials. Serum will be collected during all immobilizations for determination of PSPB and serum urea nitrogen levels. Transrectal ultrasonography was used to detect the presence, viability, and number of fetuses (Stephenson et al. 1995).

Feed samples were analyzed using sequential detergent fiber analysis to obtain estimates of NDF, ADF, and lignin. In vitro digestible dry matter using cattle innocula was also determined. The Kjeldahl method was used to determine total nitrogen (N) converted to crude protein ($6.25 \times N$). Samples were analyzed by Washington State University's Wildlife Habitat Analysis Laboratory and Colorado State University's Department of Range Science Analytical Laboratory.

We calculated metabolizable energy intake (MEI) as the product of dry matter intake, gross energy, digestible energy, and metabolizable energy. T-tests were used to test for differences in body condition relative to diet quality. Linear regression was used to evaluate relationships among diet quality, body fat, and body mass. Analyses were conducted using program SAS (SAS Institute, Cary, North Carolina USA).

JOB 2 EVALUATE RELATIONSHIP BETWEEN CALF HEALTH AND THE DAM'S NUTRITION AND BODY CONDITION

Newborn calves located by ground surveillance of cows were captured by hand. Calves were handled after >12 hours had elapsed since birth to avoid abandonment by the mother. Captured calves were equipped with expandable breakaway radio collars and numbered ear tags. Sex, body mass, total body length, and hind foot length were recorded at capture. Serum was collected and evaluated for determination of Vitamin E (α -tocopherol). In addition when available, for animals that exhibited white muscle disease, we submitted whole blood or liver samples for determination of selenium. Vitamin and mineral analyses were conducted by Washington State University's Washington Animal Disease Diagnostic Laboratory.

JOB 3 VALIDATE APPROACHES FOR DETERMINING BODY FAT AND BODY PROTEIN IN LIVE MOOSE

Captive moose on various nutritional planes and during different seasons were further evaluated for body composition. To estimate fat reserves, the rump region of immobilized moose was scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Connecticut USA) with a 5-MHz 8-cm linear-array transducer (Stephenson 1995). Ultrasonic fat thickness was measured at 2 sites along a line between the spine, at its closest point to the tuber coxae (hip bone), and the tuber ischii (pin bone). Subcutaneous fat thickness was measured with electronic calipers to the nearest 0.1 mm at the midpoint and point of maximum thickness along the line. Two fat thickness indices were further evaluated: 1) the maximum fat thickness detected along the line (MAXFAT), and 2) the sum of the maximum thickness and the thickness at the midpoint (SUMFAT). To estimate protein reserves, ultrasonic muscle thickness of the biceps femoris and gluteus medius were recorded directly under the hip and pin bones, respectively. In addition, longissimus dorsi muscle thickness was measured at the 12th/13th rib (Johns et al. 1993).

Further evaluation of bioelectrical impedance analysis to determine body composition (particularly protein reserves) was conducted in conjunction with ultrasonography. Electrodes from a plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, Michigan USA) were placed in the hindleg and foreleg of sternally recumbent moose. Resistance and conductance were recorded.

Animals were euthanized immediately following ultrasonic and BIA measurements while still chemically immobilized. Whole body mass was determined and then each animal was eviscerated and skinned (subcutaneous fat will remain on the carcass). The carcass was bisected longitudinally along the vertebral column, with one half frozen for chemical analysis. The gastrointestinal tract was emptied of ingesta (Hundertmark et al. 1994). The fetus(es) and amniotic fluid of pregnant females were removed and their mass determined to permit fetus-free calculations. Kidney fat mass was recorded as the mass, to the nearest 1-g, of trimmed fat attached to the kidney. Marrow samples were collected and frozen for determination of percent marrow fat. The entire viscera and samples of shaved hide were frozen for analysis. The frozen carcass half and visceral mass was sliced at 51 and 25 mm intervals, respectively, on a commercial band saw. The homogenate at the base of the blade was collected for each component, mixed and refrozen. Hide samples were freeze-dried and ground in a Wiley mill to

create a homogenate. Chemical analysis of frozen samples was conducted at Washington State University's Wildlife Habitat Laboratory. Crude fat was determined by ether extraction (AOAC 1975). Samples were analyzed in duplicate.

Additional samples will be used to validate existing predictive equations. Regression analysis will be used to develop additional predictive equations for body composition.

JOB 4 DEVELOP SERUM ASSAY TO DETECT TWINNING

This is a cooperative project with the University of Idaho, Department of Animal and Veterinary Sciences. A graduate student who worked closely with MRC personnel recently completed a master's thesis (Huang 1998). Serum samples collected at regular intervals in association with feeding trial immobilization enabled establishment of gestational PSPB profiles. Four cows that aborted or produced stillbirths were excluded from his analysis, but PSPB levels provide insight into the timing of reproductive failure.

JOB 5 MONITOR DENSITY EFFECTS ON BODY CONDITION AND REPRODUCTIVE PERFORMANCE

No activity on this job occurred during this reporting period with moose on natural browse at the MRC. Stocking trials will begin in October 1998.

However, considerable data from wild populations have been collected through collaborative projects during this and previous reporting periods. During March 1998 free-ranging (wild) moose in Denali National Park, Yukon Flats National Wildlife Refuge, and Togiak National Wildlife Refuge, Alaska, were immobilized from a helicopter (Bell 206B) by administering carfentanil citrate-xylazine hydrochloride with Palmer Cap-Chur equipment using 3-cc darts. Carfentanil was reversed with naltrexone. We radiocollared captured moose; we collected sera by jugular venipuncture and froze samples (-80°C) for pregnancy-specific protein B assay (Stephenson et al. 1995; Huang 1998). We used portable ultrasound to measure subcutaneous rump fat reserves and to predict total body fat as described under job 2. We also recorded the number of calves at heel during capture as a measure of cost of lactation.

RESULTS AND DISCUSSION

JOB 1 CONDUCT FEEDING TRIALS TO EVALUATE THE RELATIONSHIP BETWEEN MOOSE NUTRITION, BODY CONDITION, AND REPRODUCTIVE PERFORMANCE

Moose successfully used the Calan feed gates to obtain their feed during 1 January–30 April 1998. Appropriate height of the gates relative to the “key” collar was essential for proper functioning of the gate. The mean height above ground of the electronically active section of the door was 50.5 inches (range = 49–54 inches). Gate slot width and base height were 9.5 and 40.5 inches, respectively. The edge of the feed bowl was 13 inches behind the gate, and the maximum depth of the bowl was 22 inches below the base of the door.

Diet composition and quality differed markedly between high and low quality diets used in our trial (Table 1, 2). In vitro dry matter digestibility was 70% and 54% for the high and low quality diets, respectively. The high and low diets contained 10.5% and 6.5% crude protein, respectively.

We were able to boost aspen sawdust in the low quality diet to 45% and maintain pellet integrity by adding bentonite clay.

Dry matter intake rates calculated for the period January 1 1998–April 15 1998 did not differ ($P = 0.48$) between animals on high ($x = 563$ kg, $SE = 38$) and low ($x = 518$ kg, $SE = 48$) quality diets. However, metabolizable energy intake differed ($P = 0.02$) between the high ($x = 1482030$ kcal, $SE = 99675$) and low ($x = 1071881$ kcal, $SE = 99570$) diets. Change in percent ingesta-free body fat differed ($P = 0.03$) between moose on high ($x = -0.59\%$, $SE = 1.54$) and low ($x = -5.03\%$, $SE = 0.55$) quality diets. Similarly, change in body mass differed ($P = 0.04$) between moose on high ($x = 16.0$ kg, $SE = 8.0$) and low ($x = -17.8$ kg, $SE = 11.3$). During the trial, extremes in ingesta-free body fat ranged between a maximum of 20.6% and a minimum of 9.8%, as predicted using rump fat regressions.

We observed strong linear relationships between metabolizable energy intake and change in body fat and body mass (Fig. 1 and 2). These relationships indicate a maintenance metabolizable energy estimate of 127 kcal/kg $BW^{0.75}$ /day based on change in body mass, a value slightly less than the 134.6 kcal observed by Schwartz et al. (1988). However, a maintenance metabolizable energy estimate of 147 kcal/kg $BW^{0.75}$ /day is suggested based on change in body fat. Only 53% of the change in body fat was attributable to a change in body mass (Fig. 3).

The individualized feeding system effectively permitted measurement of intake rates for each animal in the trial. Fluctuations in daily intake rate did occur as a result of locking all animals out of feeding stations periodically to permit weighing. However, the Calan feeding system eliminated experimental bias associated with the stress of individually confining animals during feeding trials. Furthermore, experimental treatments were assigned to individual animals contained within a common pen.

For this preliminary analysis we evaluated intake and response parameters for most of the trial, rather than focusing on daily variation (e.g., intake rates). Although Schwartz et al. (1988) observed that animals on poorer quality diets compensated by eating more food and thus maintained energy intake, we did not observe this. We observed that intake rates were similar between treatments and that metabolizable energy intake was less for animals consuming poorer quality feed. The lower energy intake was reflected in greater loss of fat and body mass. One notable difference in comparing our study animals to those of Schwartz et al. (1988) is that Schwartz used males and nonpregnant females and we used pregnant females during mid to late gestation.

JOB 2 EVALUATE RELATIONSHIP BETWEEN CALF HEALTH AND THE DAM'S NUTRITION AND BODY CONDITION

Daily Calf Mass Gain

Based on measurements obtained from calf 9810, body mass remained constant between hours 3 and 22 following birth. For 2 singleton male calves (9809, 9810), daily mass gain ranged between 1.04 and 1.56 kg/day during days 1 to 5 following parturition (Fig. 4, 5). In contrast, daily mass gain (averaged from day 1 to 15) for 1 female and 2 males from twin litters was 0.60, 0.86, and 1.03 kg/day, respectively (Fig. 6). Although the measurement intervals were different,

males appeared to grow faster than females, and singletons grew more rapidly than twins. Complications associated with white muscle disease appeared to affect mass gain in calf 9809 by day 9 after birth and mortality occurred at approximately 3 weeks of age. Reese and Robbins (1994) observed that maternally raised captive moose calves in Washington gained 785 g/day. Similarly, Shochat and Robbins (1997) recorded that gains averaged 752 g/day in bottle raised calves during their first 4 weeks of life.

Vitamin Deficiency

Only 10 of 17 calves identified in utero were born alive. Furthermore, at least 3 of these exhibited symptoms of white muscle disease (e.g., weak hind end) within 3 weeks following birth. Subsequently, necropsy of calf 9809 revealed multifocal, severe, myofiber degeneration and fibrosis during histological inspection of skeletal muscle (WADDL) indicative of white muscle disease. Whole blood and liver selenium levels were 0.16 µg/g and 1.8 µg/g, respectively, in 3 sampled animals with white muscle disease (Table 3). Mean serum vitamin E (α-tocopherol) level was 0.63 µg/g (range = 0.53–0.8 µg/g).

McDowell (1992) described 2 clinical patterns in neonatal ruminants of white muscle disease, a primary symptom of vitamin E and selenium deficiencies, and we observed both at the MRC. One is a congenital form of muscular dystrophy in which young are stillborn or die within a few days postpartum. Secondly, we observed the delayed form which manifested itself at about 3 weeks of age in otherwise healthy, large, rapidly growing calves.

Flueck (1991) illustrated that whole blood selenium levels were representative of glutathione peroxidase activity in black-tailed deer (*Odocoileus hemionus*) erythrocytes. We are aware of only 1 study that determined selenium levels in free-ranging moose. Hein et al. (1994) determined that whole blood selenium levels in moose in Washington averaged 0.015 ppm (µg/g), an order of magnitude below what we observed in calves at the MRC. Selenium levels in domestic cattle are considered adequate at >0.1 ppm in whole blood. Robbins et al. (1985) hypothesized that wildlife evolved in low selenium environments and may be better adapted to them. Regardless, MRC pelleted feeds contain selenium supplemented at 0.257 ppm, a recommended maintenance level for livestock.

Although selenium levels in MRC calves appear normal, vitamin E levels at the MRC fell in the low range observed for cervids in zoos and well below the mean of 2.09 µg/g. (Dierenfeld 1989). Dierenfeld (1989) recommends that zoo ungulate feeds contain >200 IU vitamin E/kg total diet to avoid deficiencies. In contrast, current MRC moose feeds contain 5 IU vitamin E/kg.

We hypothesize that the effects of a vitamin E deficiency were manifested during this project because of the high productivity of these animals relative to previous MRC projects. In the past, although female moose at the MRC have been bred, they often were not permitted to breed year after year. High reproductive effort by females increases their vitamin and mineral costs. Furthermore, the duration (October through May) that our cows were on only pelleted feed is not typical of past studies with reproductive females at the MRC. In this study pregnant cows did not have access to browse during gestation or the first 2 weeks postpartum; thus, they were not able to consume lush green vegetation with high vitamin E in spring when supplementation at the end

of gestation and beginning of lactation may be critical. Furthermore, animals were observed consuming limited quantities of spruce, which is generally avoided by moose. Although this may be attributed to “cribbing” behavior, the terpenes present in spruce may have increased vitamin E requirements (Dierenfeld 1989).

JOB 3 VALIDATE APPROACHES FOR DETERMINING BODY FAT AND BODY PROTEIN IN LIVE MOOSE

We processed 1 surplus adult bull for body composition during March 1998. In addition to previously validated fat measurements, we were able to accurately measure longissimus dorsi and biceps femoris thickness using ultrasonography as confirmed during necropsy. Measurement of these muscles indicates potential for estimating protein reserves that may be important in energy balance.

JOB 4 DEVELOP SERUM ASSAY TO DETECT TWINNING

Thesis abstracts are presented in the Appendix. Of the 4 cows excluded from the thesis data, Laurel appears to be the only animal that terminated pregnancy early in gestation (Fig. 7). During ultrasound exams in November 1997, all 4 animals were pregnant and all but Mustard carried twins. Only Deshka carried her calves to term but delivered stillbirths. We suspect that most, if not all, of this reproductive failure resulted from the vitamin E deficiency described above.

JOB 5 MONITOR DENSITY EFFECTS ON BODY CONDITION AND REPRODUCTIVE PERFORMANCE

To date, we have collected data on fat reserves and reproductive performance from 5 free-ranging moose populations during late winter (March–early April) within the state of Alaska (Table 4). Median rump fat thickness varied between 0.9 cm and 2.8 cm in Unit 20A and the Copper River Delta, respectively. Median, in contrast to mean, values probably represent population body fat levels more accurately because of the high number of individuals with 0 cm of rump fat in some populations (e.g., Unit 20A during 1997). During early March 1997, 7 of 30 (23%) adult cows possessed <5.6% ingesta-free body fat in Unit 20A. Survival and reproductive performance of these individuals could be compromised, particularly if winter were prolonged. Although in utero twinning rates in Denali and Fort Yukon were comparable to those seen at calving, they tended to be higher than those observed at calving in Unit 20A (Boertje et al., in press). This suggests that either the accuracy of the test in Unit 20A is questionable or in utero and early neonatal losses were higher in this population. The latter may be plausible given the poorer condition of cows in this population in late winter. Testa and Adams (in press) and C. C. Schwartz (pers. commun.) observed substantial in utero or neonatal losses in moose, particularly those in poor condition. Adult cows in Yukon Flats National Wildlife Refuge possessed similar fat reserves (median IFBFAT = 7.8%) with Unit 20A, yet twinning rates were much higher. Lactational costs were higher for most cows in Unit 20A, given the higher percentage of cows with calves at heel. This and density-dependent competition for forage may have reduced ovulation and/or conception rates relative to other populations. Low fat reserves for cows in the Yukon Flats indicate winter nutritional limitation. However, high twinning rates in the Yukon Flats may indicate better summer nutrition but could also relate to reduced lactational costs as a result of high neonatal mortality. High fat reserves observed in Denali National Park and the Copper River Delta result from a combination of excellent summer and winter nutrition provided by abundant willow

(*Salix* spp.) forage and reduced lactational costs from high neonatal mortality. As expected, in utero twinning rates were high in Denali. The moderate fat reserves (median IFBFAT = 8.3%) observed on the Togiak National Wildlife Refuge are reasonable, given that animals were sampled in April, the number of calves at heel was moderately high, the population has recently moved into this area, and snow was relatively deep. Although not directly comparable, Testa and Adams (in press) observed that mean rump fat during November in Unit 13A in cows with calves and cows without calves at heel was 2.9 and 4.2 cm, respectively.

CONCLUSIONS AND RECOMMENDATIONS

Relative to the identification of a vitamin E deficiency at the MRC, we are boosting selenium levels in our feeds to 0.65–0.7 ppm. Because of a sparing interaction that occurs between selenium and vitamin E in the diet (Dierenfeld 1989), higher selenium levels in feed may aid in preventing vitamin E deficiencies as well. Furthermore, vitamin E levels in moose feeds will be boosted to 220 IU/kg.

To further develop neonatal calf age/weight relationships, we plan to continue collecting daily weights of neonatal calves during the week following birth. Bottle-raised cows at the MRC permit frequent handling of their calves.

Based on numerous successful helicopter field captures during this period, we developed the following recommendations for future projects:

1. For healthy adult cows during March, 4 mg carfentanil and 100 mg xylazine seem sufficient for prompt immobilization. If air space exists in the dart after drugs have been loaded, we recommend addition of sterile water rather than extra xylazine. If an animal is rigid while immobilized, 50–100 mg xylazine administered IV should achieve relaxation in a few minutes.
2. Dart placement is critical for rapid immobilization. Our recommended dosage is dependent upon proper dart placement in a major muscle, away from fat deposits and bone. We believe the best placement for darts is the rump, specifically at the interface between the brown dorsal hair and the black leg hair.
3. Newly manufactured brown Cap-Chur charges are adequate for darting moose from helicopters in most cases. These charges can be distinguished from the older versions by the presence of a crimped end on the brass. In our experience these are approximately equivalent in power to older version green charges, and their use will minimize trauma caused by dart impact.
4. Reversal is effectively accomplished using 100 mg naltrexone/mg carfentanil and 400 mg tolazoline. Administer both 1/3 IV and 2/3 IM.
5. In cases of severe respiratory depression, an immediate IV injection of 50-100 mg tolazoline hydrochloride is very effective in partially reversing deep sedation (effects are longer lasting than dopamine [Dopram]) but still permits completion of handling.

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Table 1 Formulation of pelleted moose rations fed at the Moose Research Center, Alaska, December 1997–May 1998

Ingredient	Feed Type (%)	
	MRC High ^a	MRC Low ^b
Aspen sawdust	25	45
Corn, ground yellow	30	25
Ground barley	29	
Beet pulp		14
Soybean meal	6.5	
Dry cane molasses	7.5	9
Dicalcium phosphate	1.1	1.1
Vitamin premix	0.3	0.3
Trace mineral salt	0.5	0.5
Protein Plus	0.1	0.1

^aOriginal ration formulated by Schwartz et al. (1985).

^bFormulated specifically for this trial.

Table 2 Mean chemical composition of pelleted moose rations fed at the Moose Research Center December 1997–May 1998

Nutrient	Feed Type	
	MRC High ^a	MRC Low ^b
Dry matter (%)	91	92
Gross energy (kcal/g)	4450	4164
NDF (%)	35.725	54.095
ADF (%)	20.035	32.5
Lignin (%)	2.74	7.925
Crude protein (%)	10.59375	6.525
In vitro DMD (%)	70.64	56.085
Selenium (ppm)	0.257	0.257
Vitamin E (IU/kg)	5.62	5.62

^aOriginal ration formulated in Schwartz et al. (1985).

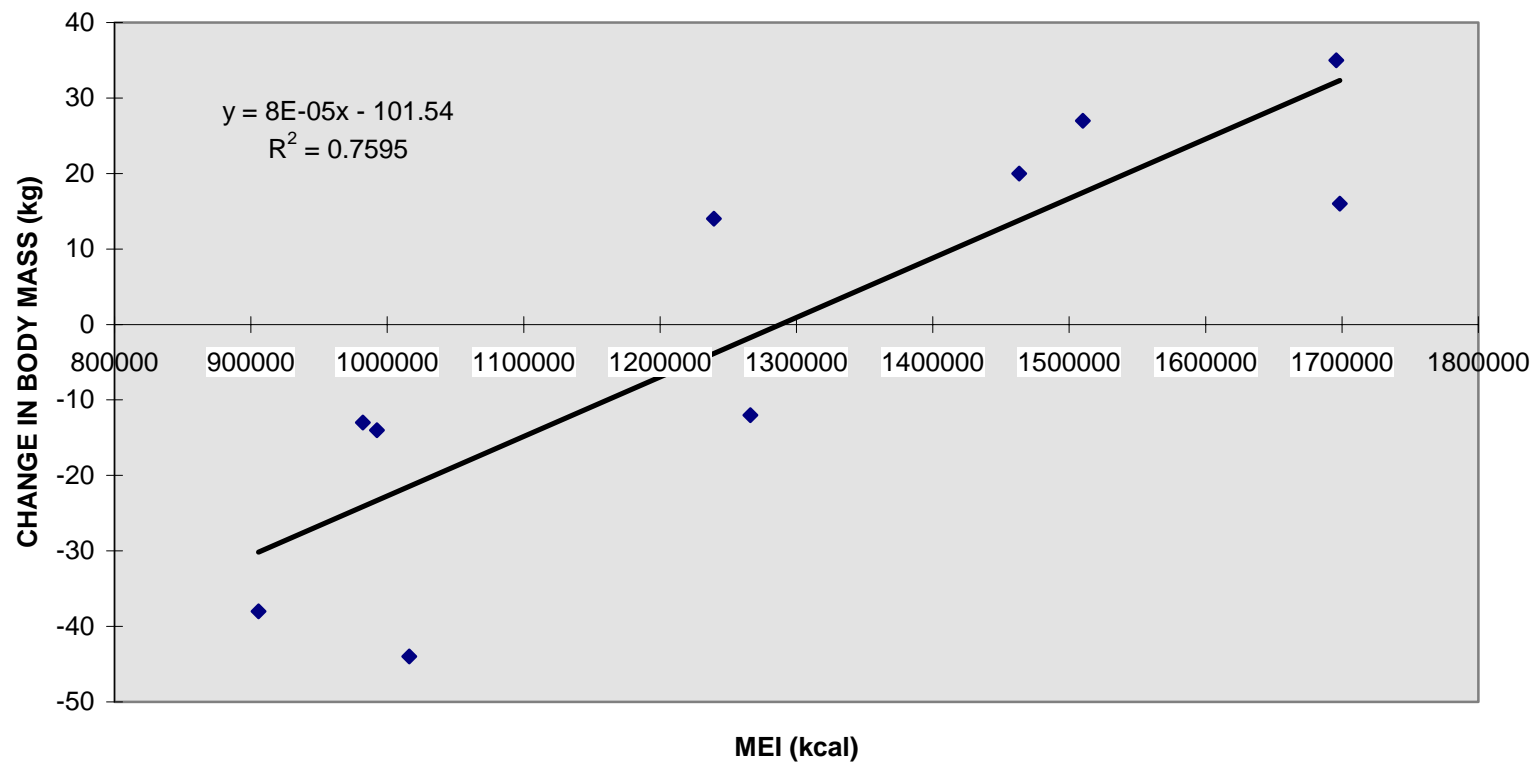
^bFormulated specifically for this trial.

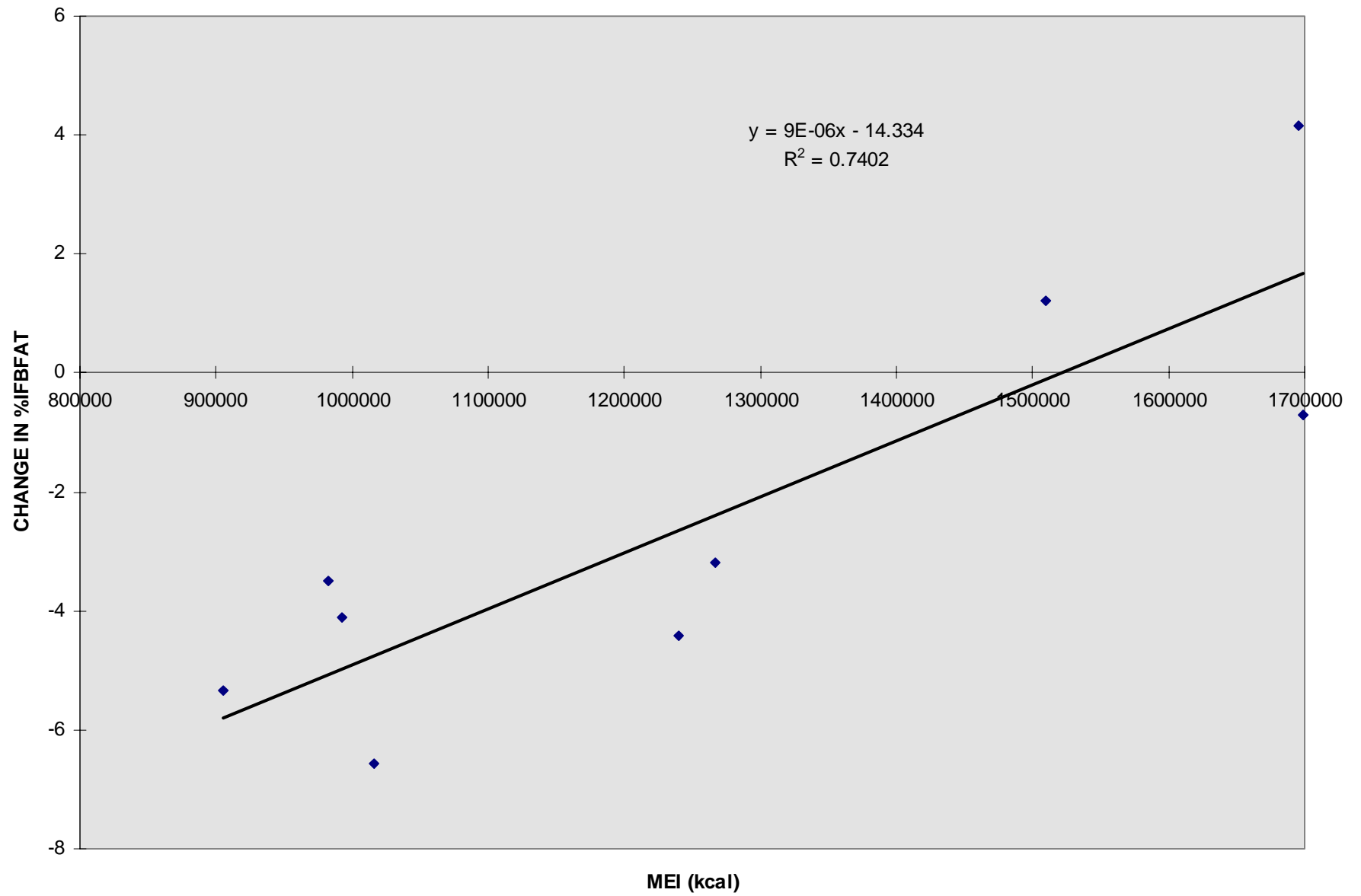
Table 3 Selenium and vitamin E levels in neonatal moose calves and the Moose Research Center, Alaska, May–June 1998

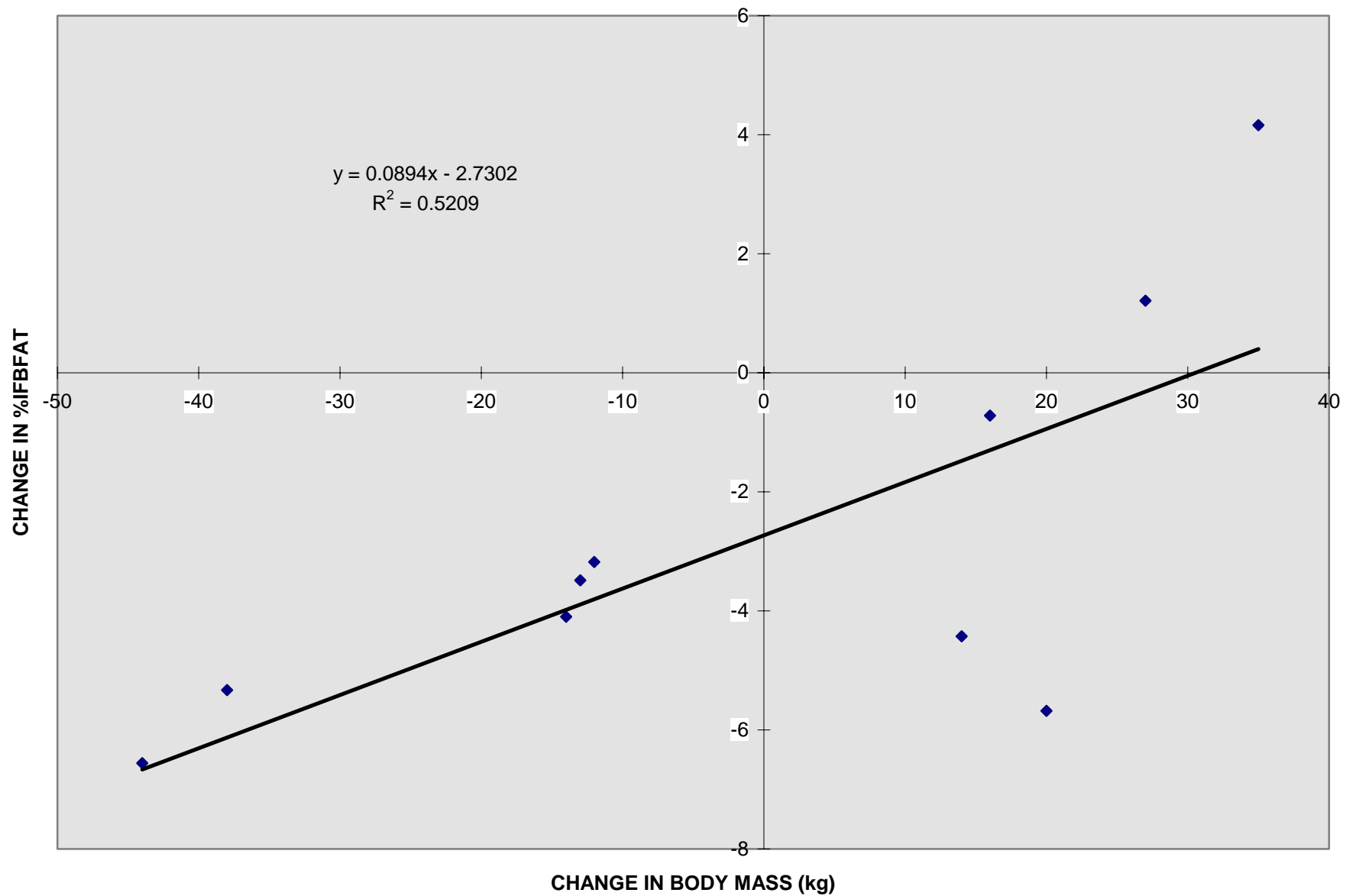
Calf ID	Dam ID	Serum Vit. E	Selenium	Fate
9801A	Erma	0.62		Mort.
9802A	Erma	0.53		Alive
9803A	Satorene	0.64		Mort.
9804A	Satorene	0.66		Mort.
9807A	Luna	0.8		Mort. (bear pred.)
9808A	Luna	0.65	0.14	Mort. (WMD, umbilical infection)
9809A	Blue	0.56	1.8 (Liver)	Mort. (WMD)
9810A	Dodger	0.56	0.18	Alive (WMD)

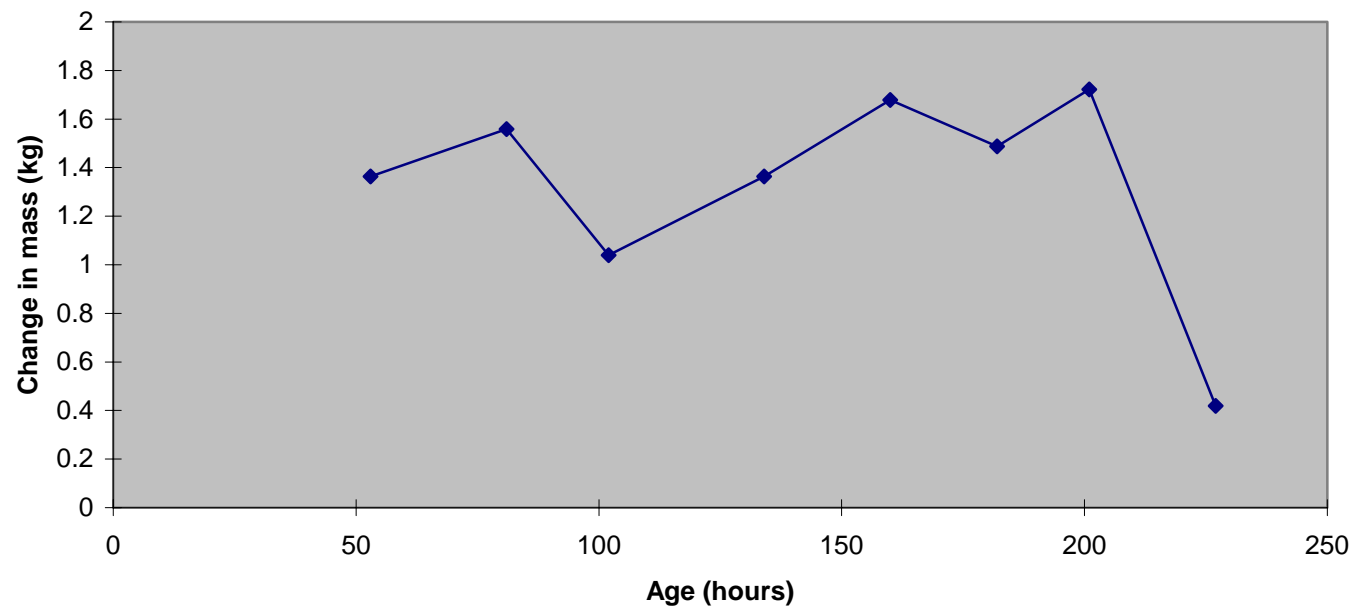
Table 4 Body fat and reproductive characteristics (calves “at heel” and in utero as determined by PSPB at date of capture) of Alaskan moose populations during 1993–1998

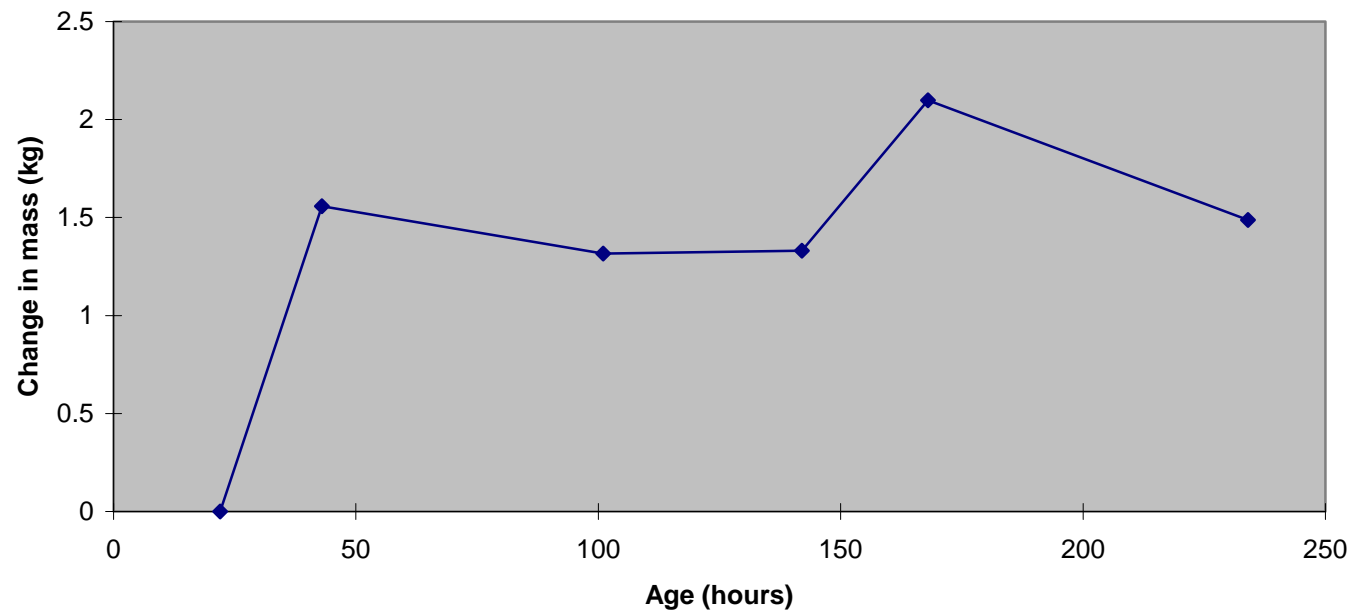
Population	Date	Median maximum rump fat thickness	Median ingesta- free body fat	Calves “at heel” (%)			Calves in utero (%)		
				0	1	2	0	1	2
CRD	Mar 1993	1.6 cm	8.9%	67	28	5			
	Mar 1994	2.8 cm	11.2%	81	19				
GMU 20A	Mar 1996	1.2 cm	8.1%	51	49		4	62	34
	Mar 1997	0.9 cm	7.4%	50	43	7	21	52	27
DNP	Mar 1998	2.6 cm	10.9%	65	29	6		40	60
YFNWR	Mar 1998	1.0 cm	7.8%	70	22	6		30	70
TNWR	30 Mar–6 Apr 1998	1.3 cm	8.3%	44	30	26		60	40

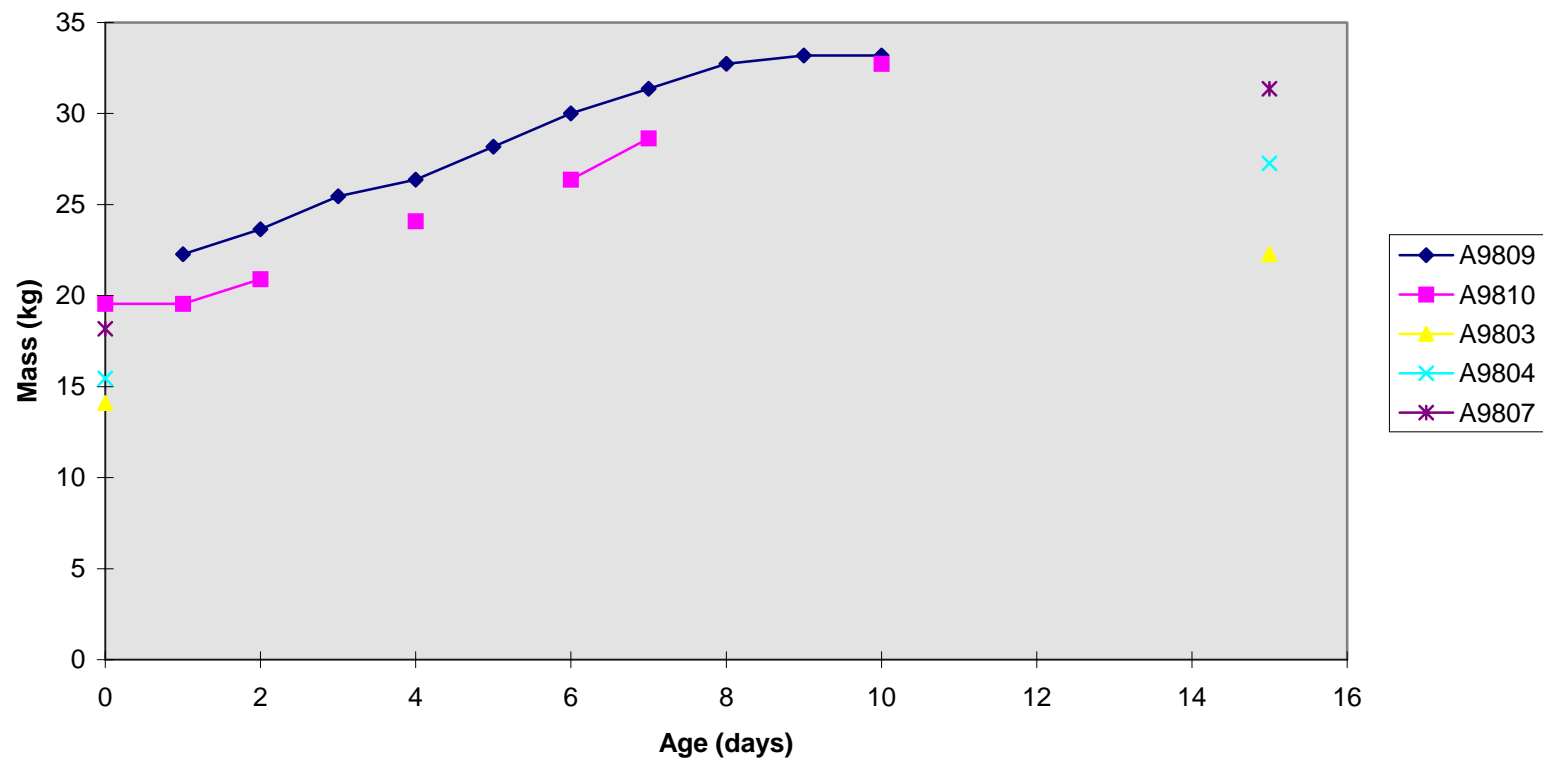


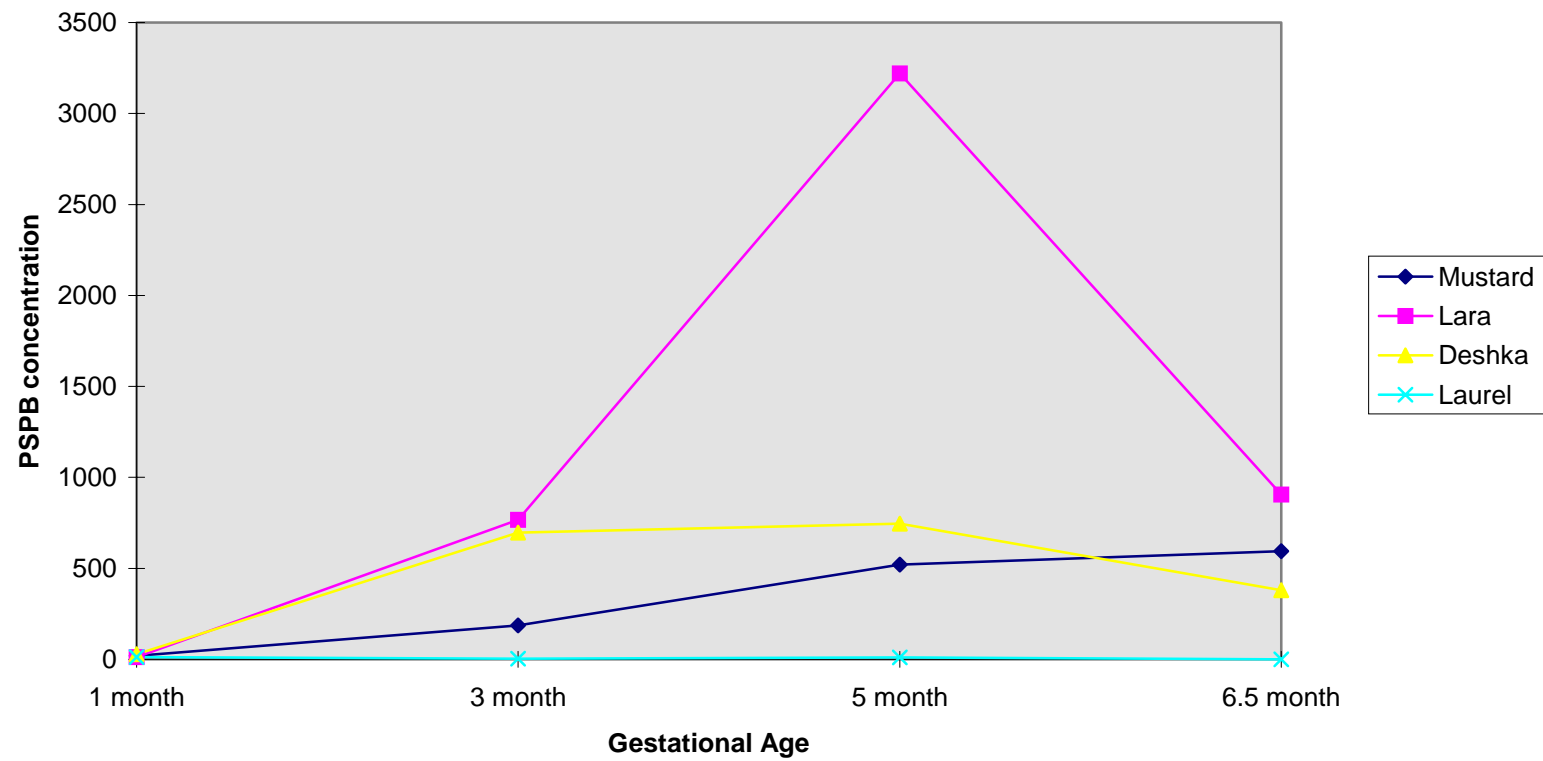


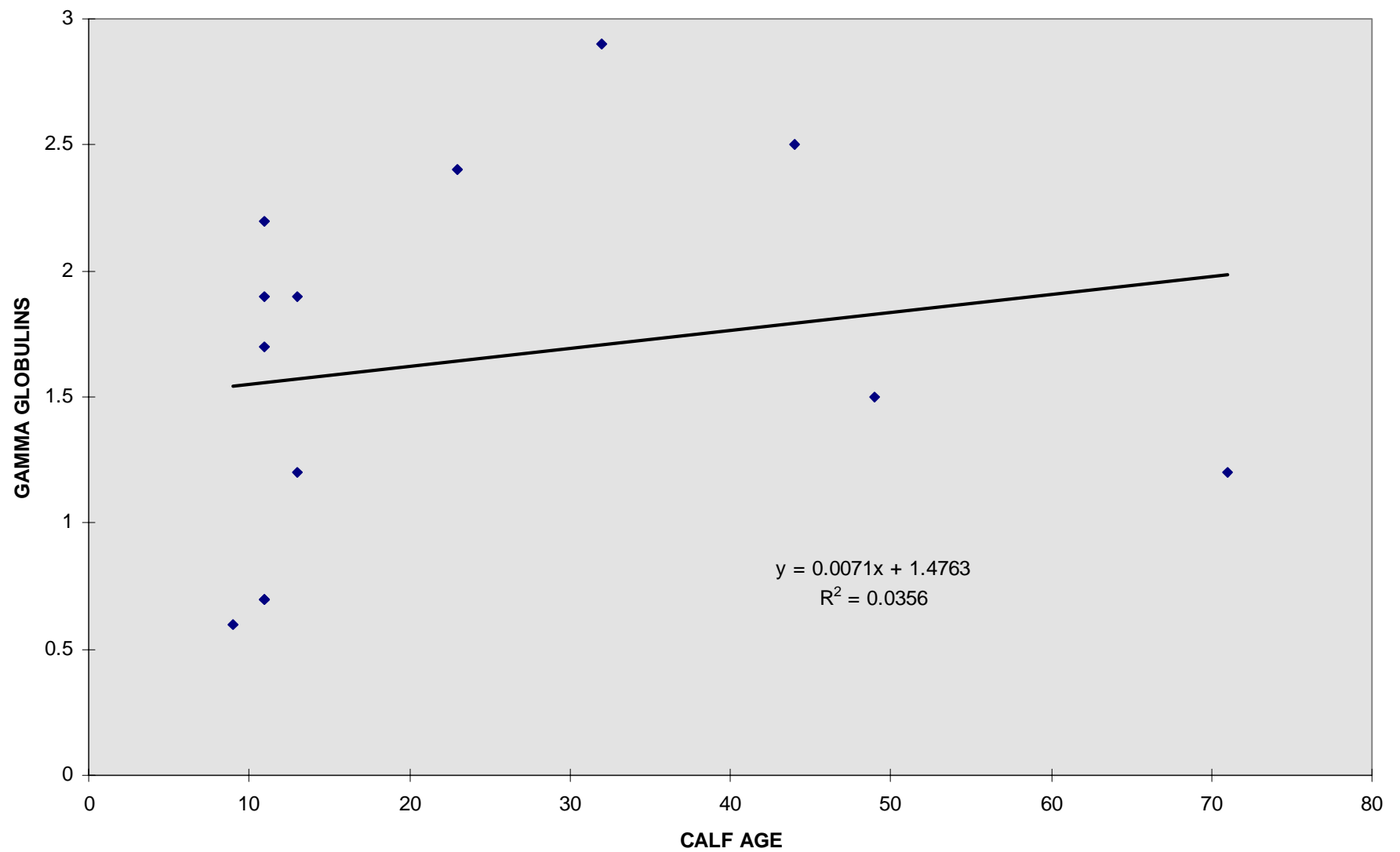












APPENDIX A. Abstracts of Manuscripts in Preparation from Fan Huang's Master's Thesis.

Isolation, Purification and Characterization of Pregnancy-Specific Protein B from Elk and Moose Placenta

Fan Huang, Diane C. Cockrell, Thomas R. Stephenson, James H. Noyes, and R. Garth Sasser

Pregnancy-specific protein B (PSPB) has been isolated, purified and partial characterized from elk and moose placenta, respectively. The procedure, which was monitored by bovine PSPB (bPSPB) radioimmunoassay, included homogenization and extraction in aqueous solution, acidic and ammonium sulfate precipitation and ion exchange, gel filtration and affinity chromatographies. The estimated molecular weights of moose PSPB (mPSPB) were 58 kD and 31 kD and of elk PSPB (ePSPB) were 57 kD, 45 kD and 31 kD by SDS-PAGE. The isoelectric points (pI) of mPSPB were 4.8, 6.6, 6.7, and of ePSPB were 4.8, 4.9, 6.1, 6.2 determined by IEF and two-dimensional gel electrophoresis. The carbohydrate content of mPSPB and ePSPB was approximately 3.15% and 4.98%, respectively. Ouchterlony double immunodiffusion test showed when recognized by anti-bPSPB, ePSPB and mPSPB shared identical and both had partial identities compared to the bPSPB. After treatment at different temperatures (20-60°C) for 1 h, the immunoreactivities of ePSPB and mPSPB in serum were very stable. Only ePSPB in serum treated at 60°C lost some immunoreactivity. After pH treatment of serum (pH 3-11) for 2 h, the immunoreactivities of ePSPB and mPSPB became lower at acid conditions, remained stable at neutral conditions and became higher at base conditions. These data show that moose and elk PSPB have properties similar to those of bovine and ovine PSPB.

APPENDIX B. A Specific Radioimmunoassay for Moose and Elk Pregnancy-Specific Protein B in Serum

Fan Huang, Diane C. Cockrell, Thomas R. Stephenson, James H. Noyes, and R. Garth Sasser

A double antibody radioimmunoassay (RIA) specific for elk and moose pregnancy-specific protein B (PSPB) was established. Sheep anti-moose (m) PSPB was used for the first antibody and placental mPSPB was used as a standard. This assay was shown to quantify moose and elk PSPB in serum. When this assay was used to detect pregnancy in elk near 40 days after artificial insemination, there was agreement with a bovine RIA at 96%. Accuracy of both RIA's was 93% compared to calving observation. Regardless of whether cows were bearing single or twin fetuses, PSPB concentration in serum increased steadily from 40 to 100 to 150 days in gestation; but near day 190, PSPB amount in serum increased slightly over the 150 day level in some and decreased slightly in others. During the different periods of gestation, the mean amount of PSPB in serum of moose bearing twin fetuses was much higher than that of moose bearing a single fetus, resulting in a significant difference in PSPB concentration in serum of moose bearing single or twin fetuses at mid-gestation. When this mPSPB RIA was used to detect fetal numbers in moose at approximately 10 weeks before parturition, a cut off point at 365 ng/ml PSPB concentration in serum was chosen to separate moose bearing single or twin fetuses. The accuracy of this detection was 90.5%. Based on this RIA, pregnancy can be detected in elk and moose and prediction of single or twin pregnancies in moose is possible. Dating the age of the fetus in moose and elk may be possible as well.